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EDITORIAL

THE PREVENTION OF SWINE FEVER

We publish in this issue of The Veterinary Journal an article of much importance in connection with the control of diseases of livestock, viz., the use of crystal violet vaccine for the prevention of swine fever. Swine fever in many parts of the world is a severe limiting factor in the pig raising industry and at times and in some localities its incidence in this country attains a considerable figure. The repercussions of its presence in herds is felt in the various restrictions governing its control under the present system and the effect on markets and the movement of pigs, generally, when the number of outbreaks attain a certain proportion may cause dislocation even in areas remote from the immediate affected premises. While there are certain recognised methods of diagnosing swine fever in affected pigs there occur outbreaks which occasion some difficulty, especially if they are not typically acute in character. We have yet to discover an entirely reliable method of diagnosing swine fever virus infection in pigs, which can be applied to all phases and conditions of the infection. For these and other reasons any method of immunising pigs against infection with swine fever virus must be examined thoroughly and, if found of value, will surely find a high place in the control of the disease.

Methods of controlling swine fever infection have, of course, been practised in this and other countries for many years. The methods have varied from a slaughter policy to the use of biological products. A successful method practised largely in America is the simultaneous injection of hyperimmune serum and swine fever virus in the form of infected blood. We understand that this method is highly successful : for certain reasons this method has not been practised in this country. The crystal violet vaccine, described in this issue, was originated in America and the results of experiments described both in America and in this country indicate very clearly that its use is followed by highly satisfactory results. It is true that the results in this country are based on experiments carried out largely in the laboratory and in a small number of herds under the immediate control of the laboratory workers. It is necessary, with all biological

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products, no matter how useful they appear to be in their effects when used under laboratory conditions, to carry out somewhat extensive trials in the field whereby its value and limitations can be judged under the various conditions of husbandry, feeding, etc., which are in common practice. We hope to see a field trial on this product. Those of us having experience of taking a product directly from the laboratory to the field know, from that experience, the importance of arriving at definite conclusions concerning the conditions under which the product may be expected to produce results similar to those obtained under laboratory conditions. It must be borne in mind, for example, that the clinical picture of an infectious disease may have features in common with that arising from an entirely different cause, so that it is very necessary to check results at various stages of an investigation. It has happened too often in practice that adequate observations in the field concerning the use of a biological product of marked and proved usefulness in the laboratory has been misused in the field, largely because an adequate field trial had not preceded its issue for general use.

There is no doubt that in this new vaccine we have a product which, when used under field conditions still to be investigated, will be found of high efficiency in the control of swine fever in this country.

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General Articles

CRYSTAL VIOLET VACCINE FOR THE PREVENTION OF SWINE FEVER

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THE introduction of crystal violet vaccine by the late Dr. M. Dorset, of the Bureau of Animal Industry, U.S.A., and the subsequent investigations carried out by his colleagues, C. N. McBryde and C. G. Cole (1936), has been noted with particular interest in this country. If the claims made for the vaccine are substantiated, it will undoubtedly provide a cheap, safe and valuable method for the control of swine fever, and one particularly suitable to the conditions obtaining in Great Britain.

Preparation of Crystal Violet Vaccine (Original Method)

"The method of preparation consists in adding one part of a one per cent. solution of phenol to nine parts of defibrinated blood [swine fever infected blood], and then adding five parts of a one per cent. crystal violet solution to 100 parts of the previously phenolised blood."

The mixture is then incubated at 37.5 deg. C. for fourteen days.

McBryde and Cole (1936) tested the vaccine on 187 pigs under laboratory conditions and on 20 pigs on a farm. A test dose of virus was given to 155 pigs three weeks after vaccination, to four pigs at the end of four weeks, and to 48 pigs after an interval of two to six months. The results were:

"Remained well after exposure	184
Slight reaction, with rapid recovery	18
Severe reaction, with good recovery	3
Severe reaction, with poor recovery	1
Died	1"

In another test on 16 pigs in which a modified vaccine containing a 2 per cent. solution of orthocresol in place of phenol was used, one animal only showed a reaction when tested with virus.

They found that the bactericidal action of crystal violet varied with the pH value of the vaccine, so in order to render it slightly more alkaline a small amount of dibasic sodium phosphate was added. The method of preparation was modified accordingly and is now as follows:

"With aseptic precautions, to 800 c.c. defibrinated blood is added, with stirring, 100 c.c. of the crystal violet solution (0.5 per cent.) and 100 c.c. of the sodium phosphate solution (3 per cent. aqueous solution). The vaccine mixture is then incubated for fourteen days at 37 deg. C."

Five lots of the modified vaccine were tested on 22 pigs; none reacted when submitted to the test dose of virus three weeks after vaccination. The vaccine was also capable of conferring a solid immunity of at least six months' duration.

Munce (1937) vaccinated 73 pigs and tested their immunity by the inocula-

tion of virus at intervals varying from 21 to 41 days later. Fourteen pigs died (19 per cent.), 13 showed severe reactions, three showed mild reactions and 43 were unaffected. In a second experiment, 47 pigs were treated with a different lot of vaccine and their immunity tested at intervals varying from 22 to 201 days by the inoculation of 1 c.c. to 50 c.c. of virus. Nine died (19 per cent.), four showed severe reactions, two showed mild reactions and 32 were unaffected. Three animals that received 50 c.c. of virus 121, 168 and 201 days after vaccination remained unaffected.

Geiger (1938) prepared three different vaccines from the blood of pigs killed at the height of the disease. One vaccine contained 5 per cent. of 1 per cent. solution of crystal violet; a second contained crystal violet with 0.1 per cent. disodium phosphate; and the third contained crystal violet and 0.1 per cent. phenol. The vaccines were used on 54 pigs. In 50 per cent. it caused swine fever, 20 pigs dying and seven recovering. Of 34 pigs subsequently tested for immunity seven died, eight became immune after light infection and nine became immune without reaction; a total of 27 pigs proved to be immune. Vaccine more than three months old was alleged to have caused swine fever in most of the pigs on which it was used. It was claimed also that vaccinated animals excreted virus even when they appeared normal.

Rubino (1938) vaccinated ten sucking pigs and tested them 22 days later by the inoculation of virus. They resisted infection.

Edgington and Schalk (1939) tested different batches of vaccine prepared in accordance with the original method. In immunity tests carried out at relatively short intervals after vaccination the results obtained were excellent.

In another experiment, 20 pigs, the progeny of immunised (serum-virus) sows, and 30 pigs from non-immunised sows, were vaccinated. Ninety days later, nine pigs from one group and 15 from the other were tested for immunity. Five of the pigs from the non-immune sows and seven from the immune sows died.

In a third experiment two types of vaccines were used; one (No. 14) prepared by Edgington and Schalk with crystal violet and phenol, and a second ("D"), obtained from the Bureau of Animal Husbandry, containing crystal violet and disodium phosphate. Thirty-seven pigs were used; 14 were injected with vaccine "14" and the same number with vaccine "D." Four from each group were tested for immunity by the inoculation of virus 42 days after vaccination and all remained normal. It was intended to test another group at the end of 90 days, but owing to the accidental introduction of swine fever the pigs were exposed to natural infection 75 days after vaccination. None died, but several showed severe reactions.

Topacio (1939) tested two vaccines, one containing crystal violet and phenol, and the other crystal violet only. The vaccine containing crystal violet alone appeared to give the best results; fifteen different lots of vaccine, each tested on one pig only, giving perfect protection against artificial infection.

De Blieck and Jansen (1939) treated five pigs with crystal violet vaccine and tested their immunity by contact with affected pigs. The vaccinated pigs contracted the disease in varying degrees of severity, but all eventually recovered.

Hupbauer and Lugomer (1940) found that the vaccine caused no undesirable

results and protected against the inoculation of virus; immunity was demonstrable after eight days and was firmly established by the thirtieth day.

De Kock, Robinson and Keppel (1940) failed to immunise pigs against an "African" strain of virus, using a crystal violet-phenol vaccine prepared from the homologous strain. The virus was particularly virulent and was apparently unaffected by seven days' incubation at 37 deg. C. There is considerable evidence that the African virus is immunologically distinct from that found in Europe and America.

McBryde and Cole (1941) reported that 342 pigs were used in potency tests of the vaccine and 98.23 per cent. proved to be adequately protected; five pigs showed sickness after vaccination, but this could not be attributed to swine fever infection. Vaccines held in cold storage for periods varying up to four years conferred complete protection in doses of 3 c.c. and 5 c.c. They showed also that holding vaccines for eight weeks at 37 deg. C. had no deleteriouus effect on potency. One sample was held for two weeks at 50 deg. C. without any appreciable reduction of potency.

Field trials were undertaken in 236 herds containing 12,572 pigs with satisfactory results. Immunity tests against either artificial or contact infection were made on 647 pigs from 166 of these herds at intervals varying from two to twelve months after vaccination. Five hundred and thirty pigs, or about 83 per cent., remained normal or showed only a slight reaction. About 12 per cent. showed severe reactions and about 6 per cent. died or were killed in worthless condition.

In view of the results of the field trials a change in procedure was made. Treatment was confined to pigs over eight weeks of age and losses decreased from 11.64 per cent. to 3.28 per cent.

A comparative test was made on two groups of unweaned pigs, one group consisted of the progeny of an immune (serum-virus) sow and a second group the progeny of a non-immune sow. The protection conferred was 61.5 per cent. in the first group and 10 per cent. in the second group.

Present Investigation

The major objects of the work here recorded were to determine the following points:

(1) The degree of immunity conferred by the vaccine against artificial and contact infection with swine fever virus.

(2) The duration of immunity.

(3) Whether vaccinated pigs are capable of transmitting infection to in-contact pigs.

(4) Whether repeated lots of vaccine of a reasonably uniform potency can be prepared.

Preparation of Vaccine

One, two or three pigs were inoculated with virus, temperatured twice daily and bled out usually on the sixth or seventh day, depending on the course of the disease. The blood from each pig was cultured immediately it was obtained; the crystal violet and disodium phosphate (or phenol) was added, and the mixture

incubated for 14 days at 37 deg. C. On removal from the incubator a sample from each flask was diluted to check the inhibitory action of the crystal violet, and then cultured. Most of the vaccines were sterile, but a few showed slight contamination which did not apparently affect their potency.

Virus

Two strains ("K" and "S") of swine fever virus obtained from America were used.

We are indebted to the Lederle Laboratories Inc., New York, for the "S" strain and to the Jensen-Salusbury Company, Kansas, for the "K" strain; these strains are immunologically identical. They gave clear-cut results and proved eminently suitable for experimental work, as no pig here throughout the course of years has survived either artificial or contact infection with either of them.

The term "no reaction" used in the text signifies that the animal had been temperatured twice a day for 14 days and had shown no rise of temperature. The majority of the vaccinated animals were not, however, temperatured, as it was impracticable, when a considerable number were under experiment, to take daily temperatures and there was, in addition, the serious objection that handling might disseminate infection. The term "remained normal" signifies that the animal appeared normal and fed well, but was not temperatured.

Test of Inactivation

Twelve lots of vaccine were given a preliminary test for "inactivation" before their antigenic value was tested.

Each vaccine was tested on two pigs. One pig was given 10 c.c. of vaccine and temperatured twice a day for 14 days. No rise in temperature was observed in any pig as the result of vaccination. The second pig was left untreated and placed in-contact with the vaccinated pig to determine whether there was any excretion of virus following vaccination. Twenty-one days or later after vaccination both pigs were inoculated with virus. All the vaccinated pigs remained normal whereas all the in-contact pigs proved susceptible and died.

This procedure, in addition to proving the safety of the vaccine, provided a severe test of its immunising value, as the vaccinated pig was subjected simultaneously to both artificial and continuous contact infection. The test virus consisted always of a 1 per cent. suspension of dried spleen tissue in 0.85 per cent. salt solution; the dried tissue was stored *in vacuo* in a refrigerator (—4 deg. C.). Both vaccine and virus were inoculated subcutaneously either behind or in front of the shoulder.

Susceptibility of Experimental Pigs

The experimental pigs bought in the open market or obtained direct from farms proved highly susceptible to the American viruses. In our experience English "field" viruses vary considerably in virulence and none has been isolated comparable in virulence with the selected American strains.

"Field" viruses usually cause a high morbidity, but the mortality varies widely with different strains. Pigs that survive may become emaciated and stunted. Susceptible pigs, held in-contact with animals clinically affected with

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"field" virus, occasionally resisted infection without showing any systemic reaction, yet on subsequent test with American virus proved immune. There was no reason to doubt the original susceptibility of these pigs, the remainder of the litters invariably proving susceptible and, as already mentioned, no untreated pig here has survived either inoculation or contact with American virus.

As regards plurality of viruses it can be said that no evidence of this has been found despite the numerous investigations carried out in the United States, and to a lesser extent in other countries, in connection with the production of immune serum and serum-virus inoculations in the field.

Period of Incubation necessary for Inactivation of the Vaccine

Preliminary observations suggested that the virus in the vaccine becomes inactivated about the third day of incubation. Starting with this information the following test was carried out.

Pig 829 was inoculated with virus and bled out on the sixth day and vaccine "23" prepared according to the routine procedure. The vaccine was tested for virulence immediately after preparation and at intervals up to the seventh day by the inoculation of pigs. (See Table.)

Numbers of Pig	*Vaccine Incubated Hours*	*Temperature Reaction*	*Result*	*Virus test of non-reacting pigs*
968	—	+++	Died 14th day	—
966	24	+++	Died 13th day	—
967	48	+++	Died 16th day	—
972	72	+	Slight reaction—recovered	—
841	72	—	Remained normal	Remained normal
847	120	—	" "	" "
848	168	—	" "	" "

It would appear that crystal violet vaccine becomes "inactivated" about the third day of incubation. As in the routine method of preparation the vaccine is incubated for 14 days, this provides a wide margin of safety. The controlling factor is probably the virus content of the blood from which the vaccine is prepared.

The possibility of a shorter period of incubation producing a more potent vaccine, as judged by size of dose and duration of immunity, is being investigated.

Establishment of Immunity

McBryde and Cole (1936) showed that immunity was not established at one week after vaccination, and that it was fully established after two weeks.

The following experiment has been carried out to obtain some further information on this point:

A group of 19 pigs of uniform size and an average weight of 70 lbs. was used. On 19 successive days a pig was taken from the group, given 10 c.c. of vaccine ("5") and placed in an isolation sty; two pigs were housed in each sty. On the third day after inoculation of the last pig, that is 22 days after the

inoculation of the first pig of the group, all the animals, with two controls, were given virus. (See Table.)

Pig number	*Dose of vaccine*	*Dose of virus Days after vaccination*	*Remarks*
671	10 c.c.	3	Died 4th day
670	"	4	Died 5th day
672	"	5	Died 7th day
669	"	6	Died 4th day
662	"	7	Sick for two days—recovered
661	"	8	Sick for three days—recovered
655	"	9	Died 5th day
657	"	10	Died 7th day
659	"	11	Dull for one day—recovered
656	"	12	Remained normal
658 etc.	"	13 to 21	All remained normal

It will be seen that most of the pigs died that were given virus within 10 days of vaccination. The pig of the 11th day appeared dull for about 24 hours, whereas the pigs inoculated from the 12th to the 21st day remained normal.

The adverse effect of vaccination on infected pigs is seen in the abnormally short interval between the inoculation of virus and death in the pigs tested three, four, six and nine days after vaccination.

The results obtained in this experiment were clear cut and much the same as those reported by McBryde and Cole (1936). Crystal violet vaccine confers a solid immunity against the inoculation of virus from about the twelfth day after vaccination.

Vaccines prepared with Crystal Violet and Phenol

The first crystal violet-phenol vaccine prepared was tested on 24 pigs, weighing from 170 to 200 lbs.

These pigs had previously been used by Dr. G. Slavin for work on necrotic enteritis (*S. suipestifer*). They had been fed broth cultures of *S. suipestifer* and all, except two, had shown high "H" agglutination titres in a test made 15 days before vaccination; tests for "O" agglutinins were negative.

Each pig received 10 c.c. of vaccine. Twenty-one days after vaccination immunity tests were carried out. The pigs were divided into two equal groups, one group was tested by the inoculation of virus and the other by continuous contact infection with six artificially infected pigs. Of the 12 pigs tested by the inoculation of virus six died and six reacted severely and recovered. *S. suipestifer* was isolated in pure culture from the principal organs of five of the six pigs that died.

Among the 12 pigs tested by contact infection, seven died and the remainder reacted severely. *S. suipestifer* was isolated from four of those that died. These pigs were in excellent condition at the time of vaccination, so whether the mortality was caused by a heavy infection with *S. suipestifer* or resulted from a vaccine of low potency is difficult to decide. The vaccine was discarded.

Vaccine "2" was tested in a dose of 10 c.c. on seven pigs and all died.

This result was so bad that a re-test was carried out on four pigs obtained from a different source and again all the animals died.

It was impracticable to titrate the virus content of the blood used in the preparation of vaccines, but in the case of vaccine "2" there was proof that the blood used contained virus, as some of it was used for the inoculation of the "virus" pigs in test of vaccine "1," all of which died in the short average time of 5.8 days.

In view of the excellent results obtained by McBryde and Cole (1936) it was decided to continue the investigation.

Vaccine "3"

Prepared from the blood of two pigs (475 and 476) inoculated with virus and bled out on the seventh day.

Test of Inactivation

Pig 524, 10 c.c. vaccine. No reaction.

Pig 500, in-contact control. Remained normal.

Seventy-one days later both pigs were inoculated with virus. Pig 500 was bled out *in extremis* on the seventh day, while Pig 524 remained normal.

The flask containing the vaccine was accidentally broken and it was impossible to carry out further tests.

Vaccines Prepared with Crystal Violet and Disodium Phosphate

It would take too much space to give details of the potency tests made on the various lots of vaccines prepared, so a summary of the results obtained with 12 vaccines is given in the following table:

Batches of Vaccine Tested	*Number of Pigs Vaccinated*	*Doses of Vaccine*			*Results*			*Virus Controls*	
		10 c.c.	5 c.c.	2 c.c.	*Remained Normal*	*Reacted*	*Died*	*Number Inoculated*	*Died*
4, 5, 6, 7, 9, 10, 11, 12, 13, 15, 20, 22.	206	132	39	34	198	3	5	80	80

As the main object of these tests was to determine whether repeated lots of vaccine of a reasonable uniform potency could be prepared, the vaccinated pigs were tested for immunity, by the inoculation of virus, between the twenty-first and sixtieth day after vaccination. Three animals showed slight to moderate reactions and five died, giving a total adequately protected of 97.5 per cent.

Comparative Test between a Crystal Violet Vaccine prepared with Phenol and one containing Disodium Phosphate

Two pigs, 1175 and 1176, were inoculated with virus and bled out on the seventh day.

The defibrinated blood was divided into two parts, to one part was added

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crystal violet and phenol (" 16 ") and to the other crystal violet and disodium phosphate (" 18 "). (See Table.)

Number of Pig	*Type of Vaccine*	*Dose of Vaccine*	*Test dose of Virus on 23rd day*	*Results*
1182	Crystal violet	10 c.c.	1 c.c.	Remained normal
1178	+ disod.	5 c.c.	"	" "
1186	phosphate	2 c.c.	"	" "
1180	Crystal violet	10 c.c.	"	" "
1183	+ phenol	5 c.c.	"	" "
185		2 c.c.	"	" "
1225	Virus control	—	"	Died 13th day
1228	" "	—	"	Died 8th day

Within the range of doses used, there was no appreciable difference between the two types of vaccines.

Preparation of Vaccine from Citrated Blood

The possibility of using citrated blood, in place of defibrinated blood, was tested, as if this method proved satisfactory it would provide a higher yield of blood and facilitate preparation of the vaccine.

Pig 996 was inoculated with virus and bled on the sixth day into sodium citrate solution and crystal violet added in the usual proportion. The vaccine (" 19 ") was incubated for 14 days at 37 deg. C.

Test of Vaccine

Number of Pig	*Dose of Vaccine*	*Test dose of Virus on the 21st day*	*Remarks*
1125	10 c.c.	1 c.c.	Remained normal
1124	5 c.c.	"	" "
1126	2 c.c.	"	" "
Control 1155	—	"	Died 9th day

Pig 1125 was temperatured twice daily for 14 days after vaccination and before the inoculation of virus, and showed no thermal reaction.

To ascertain the effect of storage on vaccine prepared from citrated blood the same lot of vaccine was retested after 408 days in the cold room (34 deg. F.).

Number of Pig	*Dose of Vaccine*	*Test dose of Virus on 30th day*	*Results*
1952	10 c.c.	1 c.c.	Remained normal
1953	5 c.c.	"	"
1950	2 c.c.	"	"
1731	virus control	"	Died 12th day

It would appear from these tests that vaccine prepared with citrated blood is as effective as that prepared with defibrinated blood. The method should permit of a more economical production of vaccine.

Vaccine prepared from an English "Field" Virus

Virus (JP) was obtained from an outbreak of swine fever in which a considerable mortality had occurred.

A suspension of infected spleen tissue in salt solution (0.85 per cent.) was passed through a Berkefeld filter (V) and the filtrate inoculated into a pig (683). This pig developed swine fever and was bled out on the eighth day after inoculation.

Defibrinated blood from Pig 683 was inoculated into Pigs 702 and 703. These pigs developed swine fever and were bled out on the seventh day and the blood used for the preparation of vaccine "21."

Test of Vaccine "21" against the Homologous Virus

Pig No.	*Vaccine "21"*	*Test dose of "JP" Virus on the 27th day*	*Results*
764	10 c.c.	1 c.c.	Remained normal
*765	10 c.c.	"	Died 13th day
766	10 c.c.	"	Remained normal
747	Control	"	Died 19th day
756	Control	"	Severe reaction Recovered

**Pig* 765. *Post-mortem examination.* Numerous petechiæ, suggestive of swine fever were present in the large colon. A saline suspension of spleen tissue (2 c.c.) was inoculated into Pig 755. This pig showed a severe reaction, lost condition but eventually recovered. It resisted later an inoculation of "K" virus. The evidence points therefore to Pig 765 having died of swine fever.

Test of Vaccine "21" against an American Virus

Number of Pig	*Vaccine "21"*	*Test dose of "K" Virus on 29th day*	*Results*
734	10 c.c.	1 c.c.	Died 7th day
736	10 c.c.	"	Died 7th day
740	10 c.c.	"	Developed into chronic case
693	Control	"	Died 9th day

Vaccine prepared from an English virus conferred no protection against an American virus.

Test of Vaccine ("5") prepared from an American Virus against an English Virus ("JP")

Pig Number	*Dose of Vaccine*	*Test dose of "JP" Virus 23 days after vaccination*	*Result*
696	10 c.c.	1 c.c.	Remained normal
699	10 c.c.	"	"
698	7 c.c.	"	"
695	5 c.c.	"	"
697	5 c.c.	"	"
700	Control	"	Severe reaction developed into chronic case

As the one virus control pig survived, the test was repeated.

Number of Pig	*Vaccine "5"*	*Test dose of "JP" Virus 49 days after vaccination*	*Result*
731	10 c.c.	2 c.c.	Remained normal
732	7 c.c.	"	"
738	5 c.c.	"	"
752	Control	"	Died 32nd day
753	Control	"	Died 10th day

Vaccine prepared from an American virus protected pigs against an English virus.

Tests of Vaccine against "Field" Virus

Material was obtained from four outbreaks of swine fever. The procedure adopted was to inoculate a susceptible pig with a mixed suspension of tissues (lymphatic glands, spleen and bone marrow) received from the field. The pig was temperatured twice daily and killed at the height of the thermal reaction and the spleen dried and stored *in vacuo* at —4 deg. C.

Each sample of spleen was subsequently suspended in salt solution (0.85 per cent.) and inoculated (1 c.c.) into two vaccinated and two control pigs; the four animals were put in a small iron sty so that the vaccinated animals were subjected to both artificial and contact infection.

The vaccinated pigs in each test were given 5 c.c. and 10 c.c. respectively of vaccine "16" (a crystal violet-phenol vaccine prepared with American virus).

Pig Number	*Method of Treatment*	*Source of Virus*	*Result*
1134	Vaccinated	Gloucester	Remained normal
1135	Vaccinated	"	" "
1154	Control	"	Severe reaction
1161	Control	"	Died 28th day
1132	Vaccinated	Cambridge	Remained normal
1133	Vaccinated	"	" "
1148	Control	"	Died 23rd day
1160	Control	"	Died 29th day
1138	Vaccinated	Cornwall	Remained normal
1139	Vaccinated	"	" "
1157	Control	"	Died 18th day
1158	Control	"	Died 14th day

The virus present in one lot of material, received from Suffolk, was so weak that the pig (1004) inoculated with it showed little evidence of infection up to the sixteenth day, and it did not appear worth while killing the animal and drying the spleen.

A vaccinated pig (1013) and a susceptible control pig (1002) were, therefore, placed in-contact with No. 1004.

The result was:

Vaccinated pig 1013, remained normal.

Susceptible pig 1002, died sixteenth day.

Pig 1004, inoculated with "field" material, died thirty-second day.

Vaccine prepared from an American virus protected seven pigs against "field" virus obtained from four English counties; six out of seven virus control pigs died.

Duration of Immunity

Test I

Ten pigs, aged three months, were vaccinated (10 c.c.); five with vaccine "5" and five with vaccine "7."

Two pigs, one treated with each vaccine, were tested for immunity on the thirty-third day by the inoculation of virus. Each succeeding month, for a period of nine months, a pig treated either with vaccine "5" or "7" was tested for immunity.

One or more control pigs were inoculated with virus at the same time as each vaccinated pig, and all died.

An untreated control pig (737) was held in-contact with the vaccinated pigs throughout the experiment and finally inoculated with virus at the same time as the pig of the ninth month.

The pigs were held in isolation and had a special attendant who did not come in-contact with other pigs. That the isolation was effective was shown by the susceptibility of Pig 737 at the end of the ninth month, as this unvaccinated control had been with the group throughout the period. All the vaccinated pigs resisted the test dose of virus without showing any reaction. The immunity test of the vaccinated pig (728) of the ninth month was severe, as in addition to the inoculation of virus it was kept in continuous contact with the control pig 737 which died of swine fever.

These pigs were vaccinated when about 60 lbs. weight; they grew well and some were over 300 lbs. when tested for immunity. The inoculation of virus caused no apparent loss of condition and the animals were sold for food twenty-one days after the immunity test.

This experiment suggests that crystal violet vaccine confers a solid immunity of at least nine months' duration on growing pigs.

Test II

Seven pigs, aged about four months, were given varying doses of vaccine ("9") and later tested for immunity, six by the inoculation of virus and one (742) against continuous contact infection.

Number of Pig	*Vaccine dose*	*Tested days after vaccination*	*Test Dose of Virus*	*Remarks*
*741	10 c.c.	158	1 c.c.	Remained normal
783	10 c.c.	210	"	"
790	7 c.c.	210	"	"
791	5 c.c.	210	"	"
784	3 c.c.	210	"	"
793	1 c.c.	210	"	"
953	Virus control	—	"	Died 14th day

*Virus control pigs 855 and 857 inoculated from the same lot of virus died on the thirteenth and tenth day, respectively.

It is of interest to note that 1 c.c. of vaccine was capable of protecting a growing pig against artificial infection seven months after vaccination.

Pig 742 was tested for immunity 195 days after vaccination by continuous contact with an infected pig (737); an unvaccinated control pig (963) was included in the group.

No. 742 remained normal, while 737 and 963 died on the fourteenth and twelfth day, respectively.

Tests against Contact Infection

Test I

Twelve pigs, 60-70 lbs. weight, were vaccinated with varying doses of different batches of vaccine. The vaccines varied considerably in age:

Vaccine "5" = 247 days
" "7" = 175 "
" "11" = 150 "
" "9" = 49 "

Two untreated pigs, 897 and 881, were held in-contact with the vaccinated pigs and were used later as "virus" pigs.

The immunity of the vaccinated pigs was tested by continuous contact with four pigs inoculated with virus. (See Table.)

Pig No.	*Vaccine*	*Dose of Vaccine*	*Test against Contact Infection Days after Vaccination*	*Results*
817	"5"	10 c.c.	96	Remained normal
804	"	5 c.c.	"	"
803	"	3 c.c.	"	"
814	"7"	10 c.c.	"	"
808	"	5 c.c.	"	"
807	"	3 c.c.	"	"
811	"9"	10 c.c.	"	"
810	"	5 c.c.	"	Died S.F. 10th day
806	"	3 c.c.	"	Remained normal
809	"11"	10 c.c.	"	"
813	"	5 c.c.	"	"
812	"	3 c.c.	"	"
897	Virus pig	—	—	Died 12th day
881	" "	—	—	Died 5th day
887	" "	—	—	Died 10th day
895	" "	—	—	Died 5th day
890	Virus control	—	—	Died 15th day
842	" "	—	—	Died 15th day
865	" "	—	—	Died 14th day

Pig 810 that received 5 c.c. of vaccine "9" died of swine fever, whereas Pig 806 that received only 3 c.c. of the same vaccine remained normal.

Four lots of vaccine, varying in age from 247 to 49 days, protected 11 out of 12 pigs, in doses of from 3 c.c. to 10 c.c. against continuous contact infection, 96 days after vaccination.

Test II

This test was carried out on 15 pigs, divided into three groups; each group

consisted of three vaccinated pigs (10 c.c., 5 c.c. and 3 c.c., respectively) and two controls.

In the immunity test one of the control pigs of each group was inoculated with virus and the other control allowed to contract infection by contact. The vaccinated pigs were thus subjected over a considerable period to continuous contact infection with two infected pigs. (See Table.)

Tested interval after Vaccination	*Number of Pig*	*Method of Treatment*	*Result*
Three months	1087	10 c.c. vaccine	Remained normal
	1085	5 c.c. vaccine	" "
	1070	3 c.c. vaccine	" "
	1075	Untreated control	Died 15th day
	1080	Virus pig	Died 10th day
Six months	1088	10 c.c. vaccine	Remained normal
	1082	5 c.c. vaccine	" "
	1073	3 c.c. vaccine	" "
	1071	Untreated control	Died 15th day
	1074	Virus pig	Died 13th day
Ten months	1086	10 c.c. vaccine	Remained normal
	1081	5 c.c. vaccine	" "
	1072	3 c.c. vaccine	" "
	1083	Untreated control	Died 17th day
	1077	Virus pig	Died 11th day

It would appear from this test that the vaccine is capable of protecting pigs for a period of ten months.

Discussion

Twenty-one lots of vaccine were prepared from American virus; four contained crystal violet and phenol, 16 crystal violet and disodium phosphate and one crystal violet and sodium citrate. A vaccine was also prepared from an "English" field strain of virus.

Crystal Violet-Phenol Vaccine

The first two vaccines gave bad results. The third vaccine was tested on one pig only; this animal subsequently resisted the inoculation of virus. A comparative test of two vaccines, one prepared with phenol ("16") and the other with disodium phosphate ("18") showed no appreciable difference, within the range of doses used, in the protection conferred.

Crystal Violet-Disodium Phosphate Vaccines

Sixteen lots of vaccine were tested against both artificial and continuous contact infection and all gave good results. The different lots were, as far as could be judged by the range of doses used, of a uniform potency.

A vaccine ("19") prepared from citrated blood protected three pigs in doses of 10 c.c., 5 c.c. and 2 c.c. respectively against the inoculation of virus. This vaccine was re-tested after 408 days in the cold room and showed no appreciable change in antigenic value.

A vaccine ("21") prepared from an English "field" virus proved of low potency, conferring no protection against a virulent strain. Three vaccines ("5," "15" and "20") prepared from American virus protected against both artificial and continuous contact infection with five English "field" viruses.

The virus contained in crystal violet vaccine becomes "inactivated" by about the third day of incubation, so the routine procedure of incubation for 14 days provides a wide margin of safety. Vaccinated pigs do not develop a temperature reaction or show any systemic disturbance. They appear incapable of transmitting swine fever to in-contact animals.

The immunity conferred by the vaccine is complete by about the twelfth day and in our experience persists under laboratory conditions for at least 10 months.

The considerable period after vaccination before immunity is established is important, as it means that in practice the vaccine must be restricted to herds free from swine fever and that animals must be protected against infection for at least 12 days after vaccination. This would appear to be the most serious limitation of the method.

The antigenic action of the vaccine is probably dependent on the virus content of the blood; in the absence of small laboratory animals susceptible to swine fever virus and the high cost of pigs, it has not been practicable to titrate the various lots of blood used.

Conclusions

(1) Crystal violet vaccine protected pigs against both artificial and contact infection with swine fever virus.

(2) The immunity lasted for at least ten months under laboratory conditions.

(3) Vaccinated pigs did not transmit the infection to in-contact pigs.

(4) Repeated lots of vaccine of a reasonably uniform potency were prepared.

Acknowledgments

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REFERENCES

De Blieck, L., and Jansen, J. (1939): *Tijdschr. Diergeneesk., 66,* 958-975. Abst. in *Vet. Bull., Weybridge, 11,* 601.

Cole, C. G., and McBryde, C. N. (1941): *J. Amer. vet. med. Ass., 98,* 454-457.

Edgington, B. H., and Schalk, A. F. (1939): *J. Amer. vet. med. Ass., 94,* 501-508.

Geiger, W. (1938): *Behring.-Mitt., 9,* 78-93.

Hupbauer, A., and Lugomer, V. (1940): *Vet. Arhiv., 10,* 214-223.

De Kock, G., Robinson, E. M., and Keppel, J. J. G. (1940): *Onderstepoort J. vet. Sci., 14,* 31-93.

McBryde, C. N., and Cole, C. G. (1936): Tested the Vaccine on 187 Pigs under Laboratory Conditions.

Munce, T. W. (1937): *J. Amer. vet. med. Ass., 90,* 307-325.

Rubino, M. C., and Tortorella, A. (1938): *Bol. mens. Direcc. Ganad., Montevideo, 22,* 352-366. Abst. in *Bull. Off. internat. Epiz., 17,* 1089.

Topacio, T. (1939): *Philipp. J. anim. Indust., 6,* 341-352.

IS AVITAMINOSIS A LIKELY CAUSE OF SO-CALLED "BROKEN-WIND" IN IMMATURE EQUINES?

By HUGH BEGG, SENR., F.R.C.V.S.

THIS question is prompted by my recollection of cases met with from forty to fifty years ago, in equines from eighteen months to five years of age. The history of one outstanding case may serve to explain the intention of this article.

A four years old van gelding was bought by a farmer in the month of May and did well until the beginning of October. He was then practically unfit for duty from breathlessness though feeding well.

Called to examine him, I found him suffering from acute emphysema of the lungs, which were markedly wheezy and the double expiratory heave was pronounced.

No green corn being available, he was given daily doses of linseed and cod-liver oils in non-bulky concentrated food.

After a few weeks idleness his condition was much worse and his oxygen hunger almost distressing to observe.

Double-rugged in his loose-box, the top half of the hinged door was left permanently open in an attempt to afford some relief.

Mild counter-irritation of the chest was tried once, lest there should be some bronchial catarrh, but it did no good.

Experimentally on three occasions different sedative drugs were given. Each dose gave relief for a day but thereafter he seemed to be worse and they were not tried further.

Towards the end of March (after five and a half months) he was greatly emaciated and in a pitiable state, though still taking a fair amount of food.

Luckily at that time the weather became mild for ten days, and he was at once put into a sheltered grass field showing new green growth. He was well clothed and kept out for eight hours per day. In seven days he showed very appreciable improvement.

By the end of April he was already heavier, and much improved in respiration. At the beginning of July he had been out night and day for two months, was in fat condition, and could have been passed as sound of wind.

I had advised that the horse should be sold back to the original vendor, but the horse bought to replace him nine months before took influenza and my old patient had to resume duty.

He kept fit until mid-November, when his old trouble became re-established and there ensued an exact repetition of the experiences of the preceding winter, though the symptoms were not so hyper-acute.

His recovery again on early grass was spectacularly rapid. He was sold back to the dealer at a loss of only £5.

Six years later a farmer stopped his milk cart at my premises and came in to ask for cough powders for a new horse. Walking with him towards the horse I saw my old patient, now showing mild signs of chronic broken wind.

I related his history and gave advice as to his food, etc., and when I saw him last he was nineteen years old—still fit for duty—still broken winded.

XUM

CONCURRENT TETANUS AND PURPURA IN A HORSE

By HUGH BEGG, SENR., F.R.C.V.S.
Hamilton

THE subject of this case was bought from a dealer to replace the horse referred to in the above article.

I was called in to examine him soon after the purchase of a new horse and found him to be suffering from influenza.

When convalescent a week or so later he was being exercised on a halter when he took fright at something, reared up awkwardly, and came to rest astride a recently cut-down hedge, sustaining a wound on the lower abdominal wall through contact with a sharp sloping stump in the hedge.

The wound had scarcely healed when he showed the characteristic symptoms of tetanus, which did not become hyper-acute, and he was always able to drink gruels, etc.

Five days later I was specially sent for and found him showing the orthodox symptoms of purpura as a sequel to his attack of influenza, while still exhibiting all the evidence of tetanus infection.

The twin infections begat an interesting picture. Legs heavily swollen to near the elbows and stifles, deep œdema of the lower abdominal wall and sheath, swollen eyelids and lips, while on the score of tetany his incisors were only one-half inch apart. His trachea lay in a hollow between two rigid sterno-maxillaris muscles, his tail still a little up and there were other signs of general stiffness of the musculature.

The message I got referred to the sudden appearance of the swellings, and I took some formalin with me.

On the presumption that desperate disease demands a desperate remedy, I at once prepared and gave him an intravenous injection of 6 c.c.s of formalin well diluted with sterile water.

Soon he began to fuss and sweat profusely, beyond my expectation, and having advised the owner to have him thoroughly and quietly dried and reclothed as soon as he settled down, I left him to it.

Next day I found that the swellings were almost completely resolved and thereafter he made a good recovery from both complaints.

I ventured the opinion that he had surely sweated well yesterday. "Sweated," said the owner, "it simply poured out of him and flowed away beneath the floor."

Review

Hoare's Veterinary Materia Medica and Therapeutics. 6th Edition. edited and revised by J. Russell Greig, Ph.D., M.R.C.V.S., and George F. Boddie, B.Sc., M.R.C.V.S. Pp. *X, 528.* Price 25s. London, Baillière, Tindall & Cox.

Our standard textbooks are by no means plentiful. We have, however, some of outstanding merit which have stood the test of time. Hoare's Veterinary Materia Medica and Therapeutics, first published in 1895, has now reached its sixth edition. Dr. Russell Greig has again edited the volume, and on this occasion Professor G. F. Boddie has joined him. Those who have been associated with them know full well that any work to which they contributed will be of high quality. In Materia Medica and Therapeutics there must be much material that has become established, but in recent times new preparations have been evolved and brought into use, e.g., barbiturates, drugs of the sulphonamide group, etc. In this edition these compounds have chapters devoted to them. It is to be expected, in the rapidly changing sequel of events following research, that even a work published in 1942 may not include all new products now in use. In this textbook, however, we are pleased to note that its up-to-date character is one of its features; few, if any, products whose value has been proved are omitted. The chapter on "Vaccine and Serum Therapy" will probably have to be revised in the next edition to include some of the most recent work, e.g., mention will have to be made of crystal violet vaccine as now used in America.

This textbook will always be a standard, and the present edition is in a number of respects an improvement on older editions.

Obituary

George William Dunkin, M.R.C.V.S., D.V.H., 1886-1942

It is with real regret that we record the death of Major G. W. Dunkin, M.R.C.V.S., D.V.H., which took place at Compton, Berks, on March 21, 1942.

After graduating from the Royal Veterinary College in 1908 he proceeded to the University of Liverpool, where he obtained the Diploma of Veterinary Hygiene. His early days as a veterinary surgeon were spent in general practice in his native town of Canterbury. In 1914 on the outbreak of war he joined the Royal Army Veterinary Corps, and in 1915 was posted

to Egypt, where in 1917 he was promoted to the rank of major, and became officer commanding the 20th Veterinary Hospital. He was mentioned in despatches. On his return to England he again practised in Canterbury till in 1923 he succeeded Professor J. B. Buxton as Superintendent of the Farm Laboratories of the Medical Research Council at Mill Hill—a position he held till 1937, when he was appointed Director of the Field Station of the Agricultural Research Council at Compton, Berks.

In Major Dunkin the veterinary profession has lost a noble member. His work in collaboration with the late Sir Patrick Laidlaw on canine distemper brought his name before the whole of the profession in this country and abroad. The painstaking work carried out by them confirmed once and for all that a filterable virus was the true cause of this common disease of dogs, and the methods they elaborated for its control are now adopted in many parts of the world. For this work alone the veterinary profession and the dog-loving public owe much to those two workers. The conditions necessary to establish the facts concerning the virus nature of the infection were largely in the hands of Major Dunkin, and the greatest credit is due to him for the careful work he carried through during the years in which the investigations were in progress. Constant vigilance was necessary to ensure the complete isolation of the animals—dogs and ferrets—throughout the whole period. It always was a pleasure to visit Mill Hill and discuss with him the various aspects of his exacting duties. Dunkin's name will for all times be associated with that great work.

At Mill Hill he also found time to study Johne's disease and Johnin. His discovery of the organism associated with Johne's disease of the sheep is but an example of the thoroughness of his methods when one remembers that several months' incubation of the cultures were necessary to produce even the slightest evidence of growth. His preparation of Johnin on synthetically prepared medium also demanded much patience.

At Compton Dunkin had many duties, for in addition to supervising and participating in the various researches, he had charge of the extensive herds of cattle, pigs and other livestock. The new laboratories were erected under his supervision, and evidence of his skill and work are very evident in all corners of the estate.

In addition to his scientific work, Dunkin took an active part in the welfare of the veterinary profession. He was a member of many committees, and served on many bodies. He received several honours from the veterinary profession—the last being the John Henry Steel Memorial Medal in 1941.

To those of us who knew him well his death has left a blank which only time will remedy. It is hard to believe that he is gone from our midst, and that we shall no more see his kindly smile or be welcomed by him when we visit Compton. He has been taken from us at a time when he could ill be spared. His memory will live with us and give us encouragement to carry on with the work in which he was so greatly interested.

William Woods, M.Sc.(Manc.) (Hon.), F.R.C.V.S., 1860-1942

WILLIAM WOODS, of Wigan, died on March 23, 1942, at the ripe old age of 81. His passing removed from our midst one of the "fathers" of our profession. Essentially a practitioner, he was known to veterinary surgeons all over the country for his ability and for his work for his profession. Many of us have passed through his hands, for he was an examiner for the Royal College of Veterinary Surgeons in Animal Hygiene, etc., from 1891 till 1937. He was also an examiner for the Diploma in Veterinary State Medicine. Woods had a kindly nature, and it was a pleasure to hear him discourse on methods of control of some of the diseases of animals which are now only names to the younger generation of veterinary surgeons in this country, e.g., glanders, rinderpest, etc. The part he played in University life—he was a teacher of veterinary hygiene, dietetics, and of sanitary law in the University of Manchester—was recognised when the University of Manchester awarded him the Degree of Master of Science. The veterinary profession also recognised his sterling value when the Royal College of Veterinary Surgeons presented him with the John Henry Steel Memorial Medal. The Profession mourns his loss. The high standard he set throughout his life will serve as an example to those who follow him.

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